

2444-Pos**Single-Molecule Analysis of Mss116-Mediated Group II Intron Folding**Krishantha S. Karunatilaka¹, Amanda Solem², Anna-Marie Pyle², David Rueda¹.¹Wayne State University, Detroit, MI, USA, ²Yale University, New Haven, CT, USA.

Group II introns are one of the largest self-splicing ribozymes found in organellar genomes of various eukaryotes such as fungi, plants and some animals as well as in bacterial genomes. Interestingly, these ribozymes can self-splice via two transesterification reactions that resemble nuclear pre-mRNA splicing in eukaryotes as they share some characteristic features with spliceosomal introns. Despite the diversity in primary sequences, group II introns show highly conserved secondary structure that consists of six domains (D1-D6) radiating from a central core. Out of six domains, D1 and D5 are the only domains absolutely essential for minimal catalytic activity, but the presence of D2 and D3 enhances catalytic activity of the intron. In order to perform its functions, large multidomain group II intron RNA must adopt the correctly folded structure. As a result, in vitro splicing of group II introns requires high ionic strength and elevated temperatures. In vivo, this process is mainly assisted by protein cofactors. However, the exact mechanism of protein-mediated splicing of group II intron RNA is still debatable.

In order to understand the mechanism of protein-mediated splicing of group II introns, we studied the folding dynamics of D135 ribozyme, a minimal active form of the yeast *ai5γ* group II intron, in the presence of its natural cofactor, the DEAD-box protein Mss116, using single-molecule fluorescence. Based on our single-molecule FRET data, Mss116 together with ATP facilitate the formation of the folded native state mainly by stabilizing on-pathway intermediates.

2445-Pos**Time-Resolved Multiphase Folding of Bacterial Group I Ribozyme**Joon Ho Roh^{1,2}, Liang Guo³, Duncan Kilburn², Reza Behrouzi², Robert M. Briber¹, Thomas Irving³, Sarah A. Woodson².¹University of Maryland, College Park, MD, USA, ²Johns Hopkins University, Baltimore, MD, USA, ³Argonne National Laboratory, Argonne, IL, USA.

Understanding of microscopic mechanism of the RNA folding process is critical for mapping out the complex mechanisms by which RNA mediates various cellular events including gene-information transfer and catalysis. Folding of RNA occurs in the presence of cations that neutralize the anionic charge of the phosphate groups, and subsequently enable long distance tertiary interactions between nucleotide bases. The process by which RNA rapidly finds its unique three-dimensional shape despite countless possible conformations remains an unsolved problem. We aim to understand the roles of electrostatic neutralization and cooperative tertiary interactions in the kinetic nucleation of structure and collapse. We have used time-resolved small angle X-ray scattering (SAXS) coupled with a stopped-flow sample system that covers sub-ms timescales to monitor the real-time structural change in the *Azoarcus* ribozyme. The collapse process was found to have at least two distinct kinetic phases within 100 ms. As the concentration of cations increases, both the kinetic constant (*k*) and fraction of fast collapsing phase increase up to $k \sim 3000 \text{ s}^{-1}$ and 80 % for high Mg^{2+} concentrations and from $\sim 2000 \text{ s}^{-1}$ and 40 % for low Mg^{2+} concentrations. This suggests that the initial charge-screening rate is crucial for determining not only the kinetic constant for the formation of tertiary interactions but also the partitioning into faster pathways. A minority ($\sim 10\%$) of the *Azoarcus* ribozyme falls into slow collapsing pathways, with $k \sim 0.03 \text{ s}^{-1}$. The results imply that the multiphase collapse process originates from an ensemble of heterogeneous structures folding on different pathways rather than a sequential step-like process. Strong kinetic heterogeneity of the collapse nucleation appeared near the thermodynamic transition, which suggests a strong competition among energetically different collapse pathways.

2446-Pos**Folding of Bacterial Group I Ribozyme in Crowded Solutions**John D. Kilburn¹, Joon Ho Roh², Liang Guo³, Robert M. Briber², Sarah A. Woodson¹.¹Johns Hopkins University, Baltimore, MD, USA, ²University of Maryland, College Park, MD, USA, ³BioCAT, Advanced Photon Source, Argonne National Laboratory, Chicago, IL, USA.

Crowder molecules in solution alter the equilibrium between folded and unfolded states of biological macromolecules. It is therefore critical to account for the influence of these other molecules when describing the folding of RNA inside the cell, which contains up to 30% by volume of macromolecules.

In this contribution we report the results of small angle x-ray scattering experiments on a catalytic RNA, or ribozyme, from the bacterium *Azoarcus* sp BH72 in the presence of polyethylene-glycol 1000 (PEG-1000), a molecular crowder

with average molecular weight 1000 Da. We observe that, in agreement with expected excluded volume effects, the presence of PEG shifts the equilibrium to favor more compact RNA structures. Two observations highlight the stabilization of the compact RNA: firstly, the transition from the unfolded to folded (more compact) state occurs at lower MgCl_2 concentrations in PEG; secondly, the radius of gyration of the unfolded RNA decreases from 76 Å to 64 Å as the PEG concentration increases from 0 to 20 % wt./vol.

We discuss several physical effects by which the crowder molecules can possibly influence the RNA folding. These include modification of ionic activity, modification of water activity, and the excluded volume effect. We present evidence that whilst all of the above effects probably change the RNA folding with respect to a dilute aqueous solution, the shift in Mg^{2+} dependence of folding transition cannot be explained entirely by the change in ion activity. We conclude that the dominant influence is likely to be the excluded volume effect.

2447-Pos**Hepatitis C Virus (HCV) - 3'UTR : a Kissing Complex Dependent Molecular Switch ?**

Sumangala S. Shetty, Mihaela-Rita Mihailescu.

Duquesne University, Pittsburgh, PA, USA.

With over 200 million people infected with the highly mutational hepatitis C virus (HCV), the need to find a conserved and functionally essential therapeutic target within the HCV genome is imperative. The 3'UTR of the HCV RNA genome, contains a 100% conserved 55 nucleotide (nt) sequence which has been proposed to be involved in interactions with a conserved sequence located within the HCV RNA coding region, interactions essential for HCV replication. Interestingly, the same 55 nt region has also been shown to be involved in the HCV genome dimerization through an unknown mechanism. In this study, we used different biophysical techniques to elucidate that the HCV RNA dimerization occurs through a kissing complex intermediate which is converted to a more stable duplex in the presence of the HCV core protein. We also provide for the first time direct experimental evidence for the interactions of the 3'UTR 55nt with the coding region of the HCV genome. Our results, which reveal dual ability of the HCV 3'UTR region to interact with both, the conserved HCV RNA sequence in the coding region, as well as with another HCV RNA genome, strongly suggest that the 3'UTR might serve as a molecular switch within the HCV life cycle for processes such as viral replication and packaging. Thus this region could serve as an extremely potential therapeutic target, allowing the inhibition of more than one process in the HCV life cycle.

2448-Pos**The Statistical Properties of Human UTRs Compared to that of Random Sequences**

Joshua S. Martin, Alain Laederach.

Wadsworth Center, Albany, MA, USA.

Untranslated Regions (UTRs) are ubiquitous controllers of genetic networks and are a key component of the cell's regulatory machinery. The molecular mechanism by which these UTRs achieve their regulatory efficacy is still poorly understood. We postulate that the RNA structure of these UTRs plays a central role in regulation. We compare the statistical properties of over 21,000 5' and 3' UTRs from the human genome of less than 1,000 nucleotides to two sets of randomly generated sequences. One set of random sequences the sequences of the human UTRs are scrambled to keep the same distribution of GC content and length. The second set of random sequences contains 1,000 completely random sequences for each combination of GC content from 0 to 1 and lengths from 25 nucleotides to 1,000 nucleotides. The scrambled sequences allow us to compare distributions of the statistical properties such as the Shannon entropy and the Helix entropy of the RNA partition function. The second set of random sequences allowed us to obtain empirical rules for predicting the average properties such as number of bases paired and average helix length of a sequence given the GC content and the length. With these comparisons it is possible to make predictions about the resilience of a given UTR to mutation and how stable a particular UTR is compared to a random sequence.

2449-Pos**Assembly Mechanisms of RNA Pseudoknots are Determined by the Stabilities of the Constituent Secondary Structures**

Samuel S. Cho, D. Thirumalai.

University of Maryland, College Park, MD, USA.

Understanding how RNA molecules navigate their rugged folding landscapes holds the key to describing their roles in a variety of cellular functions. To dissect RNA folding at the molecular level, we performed simulations of three pseudoknots (MMTV and SRV-1 from viral genomes and the hTR pseudoknot from human telomerase) using coarse-grained models. The melting temperatures from the specific heat profiles are in good agreement with the available